AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 99:493–498 (1996)

# Notes and Comments

## Technical Note: Thin Section Procedure for Enamel Histology

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A histological examination of tooth enamel has become a valuable component to paleopathological interpretations (see Goodman and Rose, 1990). Enamel microdefect analyses are most useful for reconstruction of infant, juvenile, and maternal stress, i.e., infection, malnutrition, fever, patterns among historic and prehistoric populations (see Blakey and Armelagos, 1985; Cook, 1981; Hutchinson and Larson, 1988; Lallo and Rose, 1979; Marks, 1993; Rudney, 1981; Van Gerven et al., 1990). Unfortunately, several areas of methodological confusion prevent widespread utilization of this approach for comparative data collection. These include tooth selection, light microscope technique, identification of defective developmental enamel features, and, most basic, an inexpensive and expedient means of preparing suitable dental thin sections. This technical note addresses this latter problem because detailed methodologies which permit successful replicability are seldom provided in research publications. The availability of a standardized enamel thin-section procedure will insure the most cost- and time-efficient protocol for data collection and encourage collection, synthesis, and collaboration. If preparation procedures and technical hints are not published, many researchers with access to skeletal and/or dental collections may become discouraged after failure of unguided efforts.

As most physical anthropologists initially undertaking routine dental thin sectioning do not have access to the sophisticated, state-of-the-art equipment maintained by some departments in dental and medical school laboratories, this protocol outlines material and methods that provide an adequate setup for less than $5,000. While our sections are up to three times thicker than the ultrathin sections obtained using fancier, high-tech methods, the resolution of the thicker section is identical to the latter. In fact, thicker sections are a requirement for examining developmental enamel defects (Wilson bands) using light microscopy. Sections less than 0.3 mm thick obliterate these structural features.

Ours is an abbreviated method promising replicability and excellent thin-sections derived from detailed, longer, and more traditional methods (see Marks, 1993). The technical tedium of thin-section mounting and the laborious grinding and polishing procedures are side-stepped here. While the archival quality of traditionally manufactured sections is superb, similar longevity using this new protocol remains untested. Yet, sections produced by this abbreviated methodology have remained pristine for nearly 8 years.

The destruction of the tooth by sectioning, following this method, to reveal enamel structure unfortunately renders it useless for some later studies. This fate mandates comprehensive macroscopic, casting, and photographic documentation of each tooth prior to sectioning. Dirt, glue, grease, and other residue must be removed from the crown prior to casting, photography, and sectioning. Ethyl alcohol (95%) is an effective

Received March 13, 1991; accepted August 22, 1996.

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solvent for cleaning teeth. Cleaning is followed by air drying in a laboratory oven if available, and storage in a desiccator. Many dental researchers recommend casting teeth prior to sectioning to preserve three-dimensionality. Casting provides future generations of investigators the ideal documentation. The appropriate procedures and materials, e.g., 3M Express (3M Dental Products Division, St. Paul, MN) and Coltene President (Coltene Inc., Hudson, MA), have been described in detail by Beynon (1987), Goodman and Rose (1990), Rose (1983), and Teaford and Oyen (1989).

However, casting can be expensive and time consuming, especially with large series of teeth. Given the option, according to their laboratory facilities, many researchers prefer highly detailed photographic documentation (see Goodman and Rose, 1990; Marks, 1993) using multiple views with appropriate scaling. Since the translucency and shiny surface of tooth enamel make photography difficult, the authors of one dental anatomy textbook (Jordan and Abrams, 1992) took their photographs from casts made of dental stone. Enamel translucency can be altered by applying a thin "dusting" of an opaque, matte-like material similar to the carbon-coating preparation procedure designed for scanning electron microscopy. A simple, one-step solution is to coat the crown using ammonium chloride vapor. This process renders the enamel surface opaque with a matte finish that allows oblique lighting to produce black and white photographs of exquisite surface detail (see Goodman and Rose, 1990). This procedure must be performed in a ventilated fume hood with protective eye wear. Fifteen grams of granular ammonium chloride are heated for 5 min in a test tube held by tongs until the powder begins to vaporize. Teeth are held by tweezers by the root apex or mounted in clay or styrofoam blocks and passed through the rising smoke column until the crown is evenly coated. If vapor is applied too thickly it will flake off and obscure surface detail. As the powder in the test tube begins to solidify during heating, the flame must be applied to the yet unsolidified ammonium chloride powder. Over-heating one region of the test tube will cause cessation of vaporatization and/or glass fracture. Approximately 40 single-rooted, permanent tooth crowns can be covered using 15 g of powder.

Photography should follow soon after coating as this dusted layer gradually absorbs ambient moisture and becomes translucent within several hours. After photography, teeth are cleaned and dehydrated in a sonic cleaner for 3–5 hr with 95% ethyl alcohol. Though sonic cleaning is ideal, dehydrating without this step will not substantially interfere with the embedding procedure. The teeth should be dried in a convection oven at 50° Centigrade and stored in a vacuum desiccator until embedded.

To examine the histological structure of enamel, especially dentitions from museum collections, teeth must be stabilized by embedding in plastic prior to sectioning. Since most specimens are not fresh teeth (i.e., clinical extractions or of forensic origin), the enamel has become brittle and easily fractured because the underlying dentin has lost its resiliency through dehydration and the passage of time.

A wide variety of plastic and epoxy materials are available for embedding teeth. Since infiltration of all spaces and cracks in the tooth is desired, the use of many commercially available methacrylate embedding kits is ideal. However, epoxy resins are cheaper, more convenient, and pose a lesser health hazard. Buehler Epoxide Resin (Beuhler Ltd., Lake Bluff, IL.) and Hardener kits have been used successfully by us for many years. The only criteria for choice of embedding material is that it must possess superior adhesion and not pull away from or damage the tooth and slide during curing. We find that Buehler Epo-Kwick Resin retreats or shrinks from the area surrounding the enamel crown, and subsequently pieces of enamel fall out of epoxy during sectioning. Thus, Buehler Epoxide is preferred over Buehler Epo-Kwick. Furthermore, using an out-dated hardener component of the epoxy (i.e., stored over 1 year) results in improper curing, e.g., shrinkage with eventual slide fracture, and soft, improperly cured blocks which are impossible to section. The resin remains stable and useable for many years.

Resin and hardener are mixed by weight, not volume. While ice cube trays treated

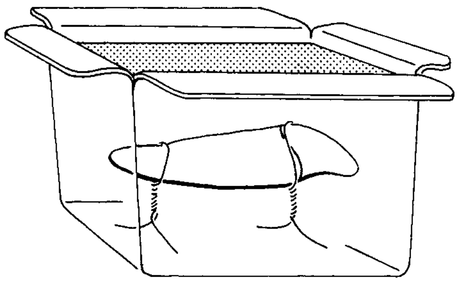

Fig. 1. A tooth suspended within a mold using copper transformer wire.

with nonstick cooking sprays make suitable molds, Polysciences Inc. (Warrington, PA) distributes Peel-A-Way disposable molds in numerous sizes. The 22 × 30 mm size holds most human teeth. Embedding medium is poured to cover the tooth only slightly, allowing for trace shrinkage during curing. Using Peel-A-Way molds, approximately 300 ml of epoxy will cover 40 human canines, while only 250–275 ml is needed (still using the 22 × 30 mm mold) to accommodate larger teeth, i.e., molars. Only 15 min is available for completing all processing steps before the epoxy begins to set up.

The tooth is supported within the mold space (prior to mixing and pouring the epoxy) by constructing a stand from thin copper transformer wire (Fig. 1). Balance is achieved by wrapping the wire around the tooth near the cementoenamel junction and twisting it beneath the tooth with two legs modeled as a brace that sit on the floor of the mold. Positioning deciduous teeth where the roots may have been dissolved or permanent tooth crowns without roots requires encircling the crown. A dab of Superglue can be applied to the wire–crown or wire–root interface to prevent the tooth from jumping out of the brace when the mold is filled. Also, a dab of cyanoacrylate Superglue (gel type works best for this) may be placed on each wire foot on the bottom of the mold for tooth stability during mold filling. An alternative is to use Superglue or to hot-glue the wire footing to a precut piece of Plexiglas that is then stationed in the bottom of the mold.

Paper identification tags are placed within the holding brackets on the inside of the Peel-A-Way molds. Only pencil and certain inks will remain legible when the epoxy saturates the paper during mold filling. *Always* test inks with the epoxy prior to mold filling as some will vanish within seconds after epoxy exposure. Also, for unknown reasons, similar brands of any color ink (including India ink), suitable at one session, may fade or vanish in subsequent trials. Finally, do not expect to read identification numbers penned on tooth roots when the teeth are embedded.

The epoxy-covered teeth in the molds are immediately placed in a vacuum desiccator and pumped down until bubbles cease to escape from around the tooth. After approximately 5 min in the vacuum, indicated by a cessation of bubbling, the molds are immediately placed into a pressure chamber under 50 p.s.i (nitrogen or air) which is placed in a convection oven for 24 hr at 50° Centigrade. Curing under pressure reduces the size of air bubbles and produces superior adhesion of epoxy onto the enamel surface and within all cracks of the tooth. Yet, while pressure curing is advised, it is not a strict requirement, and adequate though not foolproof embedding can be achieved without it. A relatively inexpensive pressure chamber can be constructed from steam pipe, iron plates, bolts, needle valve, and pressure gauge (Fig. 2). Cork automotive head gasket material (none of the other gasket material works more than one time) and vacuum grease make the least costly seal, while custom-made "O" rings, though most expensive, work best. A carrying device with several shelves and a wire handle can be made form heavy-duty screen (hardware cloth), glass or plastic tubing, and wire to fit within the pressure chamber. This should facilitate transport of 40–50 specimens (using the 22 × 30 ml molds) directly from the vacuum to pressure chamber. If an oven large enough to hold the pressure chamber is not available, the blocks can be cured at room temperature with similar success. After removal from the pressure chamber, blocks should be baked at 50° Centigrade for an additional 24 hr. The curing process is the most critical step in securing section-worthy blocks. The molds are then easily peeled from the blocks and ready to section.

Thin sectioning uses a single-blade saw

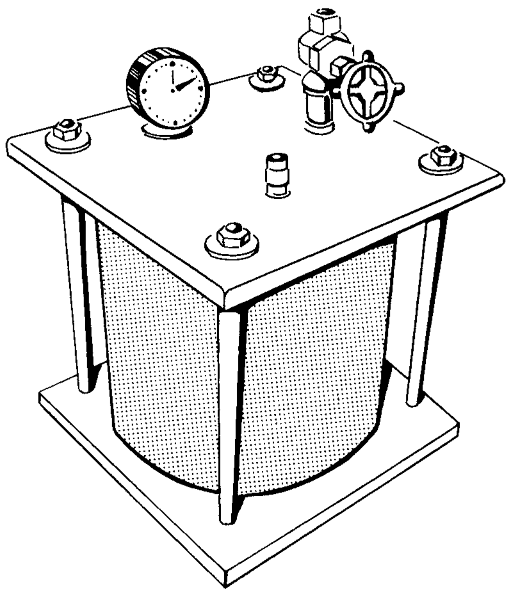

Fig. 2. Pressure chamber made from cast iron, threaded rods, and steam pipe. The pipe and rods are welded to the base plate. The lid is grooved to fit onto the top of the cylinder.

like the low-cost Buehler Isomet Slow Speed Saw. Longitudinal labiolingual thin sections, averaging 0.4 mm thick, can be routinely cut from crown midsections using an oil-cooled, high-concentration diamond wafering blade. For sections of this thickness, our Buehler saws are calibrated to a speed of 4 or 5 with a 74.5 g combination of weights. We find the 0.4 mm section the ideal thickness for preserving all enamel detail. Once the tooth is mounted, always cut two sections (in the event one is damaged during processing) because it is nearly impossible to achieve the same blade–block parallel orientation once the block is removed from the chuck assembly. Two passes with the blade are made to cut one section free from the block. Therefore, to assure two midcrown sections, the initial cut through the block is made one 0.4 mm unit to the right of the crown midline, the second cut at midline, and the third cut (for the second section) one 0.4 mm unit to the left of midline. For optimum cutting results, the blade requires dressing with a sharpening stone after sectioning four teeth. Slides ready for section attachment are scored on the back with the identification number using a diamond engraving pencil. Frosted petrographic slides, while providing superb adhesion, are not mandatory for attachment of thin sections.

This methodologically simpler alternative to traditional cutting, mounting, grinding, and polishing of thin sections not only expedites processing but avoids many unnecessary, tedious, and potentially error-laden steps. After the initial cut through the block the freshly exposed surface is cleaned with alcohol to remove cutting oil residue. An aerosol dust remover for photographic or microscopic cleansing removes debris from the prepared (already engraved) slide and the newly exposed tooth surface within the block. Several years ago, Bill Davenport discovered that rather than using permount, a traditionally utilized adhesive for thin sections, cyanoacrylate adhesives provided immediate and superior adhesion for the sections he was manufacturing. Apply three or four droplets of cyanoacrylate adhesive (Elmer's Wonder Bond and Duro Superglue have been used successfully; *never* use the gel type for this step) to the center portion of the slide adjacent to the facing surface and immediately push the slide directly onto the freshly exposed tooth surface that is still mounted in the chuck assembly of the saw arm. There are approximately 3–4 sec before the glue sets to "skate" the slide around against the exposed tooth surface and press out any small, trapped air bubbles. Press firmly about 5–10 sec to ensure a tight seal. Remember to sufficiently support the opposite side of the arm so not to bend or warp saw arm alignment. During this step too much adhesive is better than too little. Insufficient adhesive not only results in a weaker bond between section and slide, but may allow acid etching of enamel on both sides of the section.

It is important to test each tube of adhesive for crystal inclusions that will interfere with and prevent the appropriate bonding of the slide to block. Such crystals are tiny white inclusions visible to the naked eye in a single droplet. Cyanoacrylate adhesives have short shelf lives after opening, so fresh (i.e., unopened) adhesive must be often supplied.

Section thickness is achieved by using the mounted slide affixed to the block and using it as a guide for measuring the next cut. When the arm containing the chuck assembly holding the embedded tooth block (the orientation of which has never been altered) and recently attached slide is lowered, the blade will be directly under the slide. Slowly advance the slide away from the rotating blade until the blade is scraping against the surface of the slide to which the block to be sectioned is affixed. Gradually advance the section until no sound is made by the blade rubbing against the slide. After the scraping sound ceases, advance the slide one-half unit further (on the micrometer scale of the assembly arm) and this distance provides the proper thickness. After the sound ceases any desired section thickness can be chosen and measured off with the micrometer scale. Do not lower the arm containing the block and slide onto the blade soon after slide attachment since trace excess adhesive between block and slide will adhere to the top and side of the blade.

Observations of Wilson bands and other enamel features require acid etching to differentiate enamel prism orientation. Wilson and Shroff (1970) and Rose (1977) recommend sections be etched in one normal solution of hydrochloric acid for 15 sec with moderate agitation of the slide. After etching, rinse slides in tap water (do so thoroughly or acid etching will continue), dip in 95% alcohol, and air dry. Wright (1990) found that etching in hydrochloric acid produced a precipitate on the enamel surface when observed with a scanning electron microscope and chose to etch with a 0.074 molar solution of phosphoric acid. In another SEM study, Marks (1992, following Rose, 1977) found no such precipitate. If sections are vigorously agitated for the entire submersion period and thoroughly rinsed, not simply dipped into the acid solution and water, a precipitate will not accumulate on the enamel surface.

In the past we polished sections with three grades of diamond paste to remove scratches produced by the saw blade. We have since discovered that etching alone removes sufficient enamel to eliminate all scratches produced by the diamond blade, and thus eliminates the laborious necessity of polishing sections for enamel inspection.

Though our revised method alleviates many possible sources of damage to the tooth, thin section, diamond blade, and saw, we reiterate that two thin sections should be manufactured from each tooth in case of curation mishaps. Our intent has been to provide details and technical tricks developed from dental thin-section production in a minimally outfitted laboratory. These procedures and products will provide, with some practice, high-quality dental thin sections with few losses coupled with a marked decrease in production time. They are not offered to replace those successful, well established procedures performed by experts with more advanced laboratories but to encourage newcomers operating under perhaps greater budgetary constraints to approach this sort of analysis with less methodological anxiety.

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